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13. ABSTRACT *(Maximum 200*
During this first year we have made progerss in three areas: 1) design of the exercise ergometer and development of a reliable experimental protocol, 2) validation of MRI as a viable method of measuring differential muscle recruitment in a local system (the lower leg with plantar flexion), and 3) development and implementation of a total body MR imaging protocol that enables the identification & differentiation of muscles that are recruited by our experimental protocol. In area 1 we have successfully implemented an experimental protocol that is reliable in a male population, we have not yet tested our protocol in a female population. We may need to reduce the mass of our weighted box in both populations to ensure that the female subjects can complete the task. In area 2 we demonstrated that MRI provides a viable tool to assess differential muscle recruitment patterns. In area 3 we have developed and implemented an echo planar total body MR imaging protocol that reliably measures total body muscle recruitment during our experimental protocol. We have successfully completed a study with a male subject and have been able to identify differential muscle recruitment patterns. Although we were not able to study the number of subjects that we had initially hoped to study, the experimental protocol and MR techniques are ready. With the addition of a staff member to recruit subjects, we are currently screening subjects and preparing them for studies on a weekly to bi-weekly basis.

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H. B. Poe 10-21-97

PI - Signature Date

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INTRODUCTION

The first year of this study has been focused in three areas: 1) Design and implementation of the experimental protocol, including the exercise ergometer and the exercise protocol, 2) Validation of differential muscle recruitment within a specific muscle group as a function of joint orientation, and 3) Development and implementation of a total body MRI protocol that enables identification of muscles recruited by our experimental protocol, including data acquisition and image processing. The experimental protocol is intended to simulate conditions that might be encountered by troops in the field and compare male & female responses. An exercise protocol was therefore designed to challenge test subjects with a standard military lift & carry task, at a rate that might be encountered in the field (3 lifts per minute), over a prolonged period (3hr of exercise in a 6hr period, or 540 total lifts). In the area of our basic experimental protocol, the ultimate purpose of this first year, to design and implement an exercise protocol that, while it can be accomplished, significantly challenges both male and female subjects. This purpose is currently incomplete as we have yet to establish the protocol in a female population.

In our second area of focus, we contend that in order for total body MRI / muscle recruitment data to be generally accepted in the scientific community, MRI needs to be well established as a technique for measuring differential muscle recruitment patterns in a local region. We performed a scout study to validate differential muscle recruitment within the lower leg. While it is generally accepted that the three major muscles that contribute to plantar flexion are recruited based upon the orientation of the knee (1,2), this has not been specifically demonstrated with MRI techniques. The two heads of the gastrocnemius are primarily recruited when the knee is fully extended (180°) (1,2), and the soleus is thought to be primarily recruited when the knee is fully flexed (≤90°) (1). At intermediate degrees of knee flexion/extension both muscles are thought to contribute (1). We studied recruitment of the medial & lateral gastrocnemius and the soleus under different conditions of knee flexion/extension in order to build a foundation for the total body MRI studies that will comprise the first specific aim of this four year project. Our protocol of plantar flexion at different knee angles is currently being reproduced at the University of Miami using EMG with the same preliminary results. The purpose of this portion of our first year, to validate MRI is partially accomplished in that we need to acquire more data points. However, the preliminary results are quite convincing and we feel that with 5-7 more studies both here and in Miami, we will have a solid collaborative publication in the field of

biomechanics. While this portion of the study did not recruit subjects under the U.S.Army protocol, nor was it an official part of the proposed project, it is absolutely necessary as a preamble to the data that will be reported later; therefore, it is included in this report.

Recently a total-body MRI protocol was developed by Johnson, et al. that employs echo-planar technology (3). The technique allows 180 images to be acquired in as little as 18 seconds. Dr. Johnson, who is here at Yale, is now collaborating on our project, and we have spent much of this year refining his technique for application within the design of our experimental protocol. By incorporating the total-body protocol into our experimental design we can now study exercise induced T_2 changes throughout the bulk of the body's musculature. However, because subjects are in and out of the imager 13-14 times over the course of a single experiment, subject positioning is critical so as to minimize potential problems with co-registration. Another drawback to this new technique is the bulk of data that is generated in each study (in excess of 9000 images, or about 1Gb of data). This has necessitated a re-thinking of how we handle our data storage and image processing. The purpose of this portion of our first year, to establish a viable and reproducible MRI protocol that identifies recruited muscles, has been accomplished and we have successfully completed a study with a male subject. Based upon this initial study, the preliminary indication is that the muscles of the forearm are most consistently and heavily recruited with our experimental protocol. The final purpose of our first year, to make a decision about what is the primary recruited muscle, is therefore not completely accomplished. While we have an idea about the most heavily recruited muscle, we need more data points and data from both male & female subjects before a final decision can be made.

During this first year we have restructured and rebudgeted this contract in order to streamline the experimental protocol and generate some support for purchase of equipment. These changes in scientific structure will seen during these next three years, and will allow us to obtain more data from fewer studies. The savings in experimental costs will be reinvested in equipment that will improve our data acquisition and processing capabilities. We will add one more part time staff member to function as our subject liaison, the exercise physiology position has been changes to a computer engineering position. This report will acknowledge the original statement of work (SOW) and address the revised SOW.

This first years work has been based upon a body of literature that demonstrated exercise induced signal changes on MR images (4-7). These changes are affected by factors that include exercise intensity (5,6,8-10), duration (6,9,10), and type of exercise (11,12). The knowledge that muscles working at a greater intensity for a specific duration will exhibit a greater signal intensity than those working for the same duration at a lesser intensity provides the basis for this work which looks at a number of muscles working

simultaneously at different workloads. The experimental protocol that we have developed is based upon the systems employed at the USARIEM in Natick, MA. and were constructed in collaboration with Dr's. Harmon and Frykman. The strength of this first years work has been the combination of echo planar MRI (3) with an exercise protocol that simulates a standard military lift & carry task. The technical development that has been completed this year leaves us in a position to carry out our proposed research aim of comparing muscle recruitment in men and women during the performance of this task.

BODY OF REPORT

A. Experimental methods, assumptions, and procedures:

1. Design & implementation of the experimental protocol - A repetitive lifting exercise ergometer was constructed from a steel roller assembly of the type routinely used for moving boxes into a warehouse (see Figure 1). Because we have no need to perform at lifting rates greater than five lifts per minute, we used a single 10ft section of roller. The roller was mounted on a frame such that it reached a height of 52in at one end and 5in at the other end. A platform was attached at the lower end to provide a landing. The entire assembly was mounted on locking rollers for mobility. A box (12in X 12in X 16in) was constructed of high molecular weight plastic (1.5in thickness) with handles mounted at 45° angles (see Figure 2). The box weight (45lbs) was augmented by mounting circular weights with a threaded nylite rod (see Figure 2). The exercise protocol to be used with this ergometer consists of three lifts per minute of the box with 20lbs of additional weight (total weight = 65lbs), and will be the same for both male and female subjects. The protocol is 3 lifts per minute for 15min (45 lifts) followed by 15min for data acquisition, during which the subject is allowed to rest. There are 12 sets of exercise (12 15min periods, 540 lifts) over a 6hr period (3hr of exercise). With each lift the subject, from a squatting position, lifts the box (65lbs) from the lower end of the ergometer (5in) and carries it (about 10ft) to the upper end of the ergometer (52in) placing it on the rollers. The subject then walks back to the lower end of the ergometer and repeats the process. For subject recruitment & screening we designed a set of forms for each subject to fill out giving a diet & exercise history. We also designed a checklist to be followed during the month prior to each study. These forms are presented in Appendices A,B, and C. Data from these forms & checklists is entered into a MS Access based computer data bank.

2. Validation of differential muscle recruitment - Exercise induced T_2 changes were compared in two different muscles (gastrocnemius, soleus) that were worked at a constant and standardized workload for a fixed amount of time (5min). The muscles worked against a constant resistance equal to 25% of the measured maximal voluntary contraction (MVC) for the gastrocnemius (2). An exercise ergometer that has been employed previously (6) was used to enable dynamic contractions that rotated each muscle through its full range of motion. The ergometer, which mounts directly to a standard GE Signa patient bed, provides resistance via a sealed dual-chambered pneumatic device (6). With the rear chamber pressurized to 14-26 pounds, in a circuit that included a tank of approximately 100 times the volume of the cylinder, the resulting mass to be moved by either the gastrocnemius or the soleus was between 45 and 80 pounds depending upon the subject, corresponding to a normalized workload of 25% of MVC. The foot was placed in a pedal that articulated with the air filled cylinder such that work was performed at the ball of the foot by rotation of the foot about the ankle. We studied three healthy subjects (2M,1F) age 20±1yr, weight 78±8kg, and height 174±5cm. Each gave informed written consent according to our protocol #6639 approved by the Yale University Human Investigation Committee.

Each subject performed the same exercise protocol (5min exercise @ 25% of MVC) on three different occasions with the knee at three different degrees of flexion / extension: 1) plantar flexion with the knee fully extended (180 degrees), 2) plantar flexion with the knee flexed at 90 degrees, and 3) plantar flexion with the knee at an intermediate extension (135 degrees). A single resting MR image was obtained prior to each exercise and another scan was taken immediately after each exercise. Since subjects performed multiple protocols on a single day, contralateral legs were exercised in such a way that the same leg was never exercised without a minimum of 40min rest period. Dynamic plantar flexion MVC values were determined from a standing position on a counterbalanced scale with handles installed. MVC values ranged from 220lbs to 320lbs.

Single slice MR images were obtained on a 1.5T GE Signa system (General Electric, Milwaukee,WI) at 63MHz. Subjects were positioned supine within the imager with an extremity coil positioned midcalf and with their foot positioned within the foot pedal assembly of the exercise apparatus (6). Transverse calf images (0.5cm slice thickness; FOV = 20cm; 128 X 256 matrix; NEX = 1) were obtained using a multiple spin-echo sequence (TR = 1000msec; TE = 30,60,90,120msec; total scan time = 2min20sec). T_2 values before and after exercise were calculated in a region-of-interest (ROI) within both exercised and non-exercised muscle. Each ROI was selected so that visible blood vessels

and fat were avoided (6). In exercised muscles 4-8 ROI's (0.8cm X 0.8cm X 0.5cm) were selected in order to compare the uniformity of T_2 increase throughout the muscle. T_2 values were calculated in the peroneus, the medial and lateral heads of the gastrocnemius, the soleus, and the anterior compartment (anterior tibialis and EDL) by fitting four data points (each echo) to a monoexponential decay curve using a least-squares algorithm. Mean T_2 values with variances were calculated and compared with a paired student t-test.

3. Development and implementation of total body MR imaging - Total body scans were obtained using a General Electric Signa 1.5 Tesla instrument, version 4.8, outfitted with resonant gradients for echoplanar imaging (Instascan, Advanced NMR, Wilmington, MA). The "autotune" utility on Instascan was used to optimize the timing of the sampling of the echo. Shimming was performed if necessary using the first order room temperature shims. The shoulder level was used for this purpose.

Patient table movement was begun using the "table rocking" utility found under the Signa utilities/tools menu. The table was not actually rocked back and forth; instead a long excursion was prescribed, with a long end-of-excursion pause. The result was that the subject was swept through the magnet in one direction, and the imaging was continued until the table paused. Then the table rocking was disengaged. A typical total table excursion was 1800 mm, and end-of-excursion pause 10 seconds. Table speed was 20 mm/sec. Imaging began at the top of the head and continued down to the ankles. The total number of images was 200 per sweep. Three sweeps were performed one immediately after the other, at TE = 60, 30, and 60 msec respectively. Total imaging time was 90 seconds for each sweep. The complete set of images was obtained within about 12 minutes after the end of exercise.

The body radiofrequency (RF) coil was employed. In all cases, a single shot spin echo echoplanar pulse sequence was used. All images were one signal average obtained one at a time at isocenter. Since the table was moving, this resulted in a stack of axial images from head to foot. Slice thickness was 10 mm, slice overlap was 0 mm, and field of view 40 cm by 20 cm. The acquisition matrix was 128 by 128, with the read direction interpolated to 256 in postprocessing. The time between RF pulses was 500 msec, selected using the "TR" parameter on the console; note that since the entire image is obtained in a single shot, and since the slices do not overlap, the actual TR is infinite (except for effects of an imperfect slice selection profile).

Exact subject re-positioning was essential to ensure optimal registration each time the subject was placed in the imager. The subject's feet were placed in a form constructed of foam so that the two lower legs were positioned the same distance apart each time the

subject was re-positioned. The subject was given a Lexan rod to hold with his/her hands so that the hands & arms would be held in the same place with each scan, then the subject's upper body & trunk were strapped to the patient bed with a cloth mesh & velcro restraining device, and the subject's knees were bound with a velcro strap. During the initial image pass the patient bed was marked with arrows drawn on tape with a Sharpie, that corresponded to specific anatomical points (ear, elbow, knee) on the subject. During each subsequent re-positioning the subject was placed on the patient bed in alignment with those anatomical markers and strapped in position to prevent movement. The entire re-positioning process required about a minute. The subject was then landmarked, using the electronic alignment hardware on the imager, and placed in the imager.

After the completion of the exercise and MRI data acquisition protocol, three stages of data processing are performed to determine muscle activation. These stages have been defined as image reconstruction, T_2 map calculation, and T_2 map sampling. Each of these stages are described below:

Image Reconstruction

Due to time constraints during the imaging phase of the exercise protocol, the MRI data is stored in the scanner's computer system in its raw, Fourier domain (K-space), format. This data must be processed via a sequence of steps to reconstruct spatial images of the study subject's body. The first step in this sequence is the transfer, via a local computer network, of the raw data from the scanner computer to Unix-based workstations (either Sun or SGI computers), located in the NMR research computer lab. From these computers, the raw data is archived to digital tape to provide long term storage and to reduce the hard disk storage requirements. The amount of raw data is massive: each raw file contains all of the data for one complete imaging pass through the body (i.e., 180-200 images), each consisting of a matrix of size 256 X n of four byte data values, where n is usually 84 or 114, depending on the TE value used during imaging. Thus a single raw data file can require over 23Mbytes of storage space; given that a single experiment can generate 40 such data files, the data handling and processing is very demanding, both in computer and time resources.

Once the data has been transferred, the next step in image reconstruction is removal of noise from the raw data. The imaging protocol used to collect this data tends to produce occasional "speckle-type" noise (or spikes) in the resulting K-space (frequency domain) data. These spikes must be removed to eliminate striping artifacts in the spatial domain images. The noise removal is achieved using a non-linear filter, similar to a top-hat transform, that detects isolated pixel values much greater or smaller than the values of its neighbors; these spurious values are replaced by an average of the surrounding pixel

values. Since the K-space data is complex, the real and imaginary component matrices are processed in two separate passes of the filter.

Following noise removal, the K-space data is converted to spatial domain images via a Fourier transform process. Due to the short TE times used in the imaging process, complete K-space data cannot be acquired. To reconstruct the partial Fourier images (PFI) without a large loss in resolution, it is preferable to invoke Hermitian symmetry to fill in the missing K-space data prior to Fourier transformation. However, spatially dependent phase shifts, produced by the scanner hardware, can destroy the symmetry of the data. To restore the data symmetry, a 2D version of the Fourier correction algorithm described by McFall, et al., (13) is used. This algorithm estimates the phase correction factors for the partial K-space data using a Fourier transform process applied to the low frequency portion of the collected data. Once the phase factors are computed and applied, restoring the symmetry of the collected data, Hermitian symmetry is used to fill in the missing data. Finally, a Fourier transform is applied to the full K-space data to produce the spatial domain image.

The entire reconstruction process, including the denoising filter, has been implemented in Matlab (The MathWorks, Inc., Natick,MA). Processing of 150-200 images from a single raw data file requires approximately two hours on an SGI IMPACT computer equipped with an R10000 cpu and 128 Mbytes of RAM. The resulting images consist of 256 X 128, two byte pixels; all images from a single raw file are stored in a single file, in a format compatible with the ANALYZE software package (Biomedical Imaging Resource, Mayo Foundation), in preparation for subsequent processing. These images are stored on magneto-optical disks when not needed for further processing.

T_2 Map Calculation

A T_2 map is a matrix of T_2 values, with pixel-wise correspondence to the processed images, computed from the three body scans acquired during a single imaging time point. As this computation assumes that corresponding image pixels represent the same volume of tissue in the body, the images are checked visually, via a color overlay process, for proper alignment. Since much effort is taken during the imaging process to restrain the subject, very little motion has been observed between body scans in the data acquired to date.

T_2 values are computed by fitting an exponential function to the values of corresponding pixels in each set of three images, one image from each scan, given the TE values used during the data acquisition [$S(TE) = S(0)\exp(-TE/T_2)$]. Prior to fitting, pixel values are thresholded to detect pixels below a specified signal-to-noise ratio; T_2 values for below threshold pixels are set to zero. The T_2 values are scaled to preserve sufficient accuracy for storage as two-byte integers in files having the same format as the

corresponding image files, facilitating T_2 map sampling. This processing has been implemented in Matlab.

T_2 Map Sampling

Muscle activation status was determined by computing changes in T_2 values for selected muscles between pre- and post-exercise imaging sessions. Identification of the image regions corresponding to the muscle of interest is achieved using ANALYZE, an interactive image processing and visualization package; this software enables a user to define regions of interest (ROI) in an image by tracing region borders with the mouse. Region definition is a time consuming process as a particular muscle extends over several image planes. Due to changes in subject positioning in the scanner between imaging sessions, the location of the muscle varies across image sets, requiring each muscle of interest to be defined in each image set. Muscle region identification can be done using the original images or the corresponding T_2 maps.

Once the ROI's are defined, the T_2 values for the pixels within the selected regions are sampled. The T_2 values are thresholded to exclude values much greater than or smaller than physiologically possible values. The mean and standard deviation for values within the range are computed for each ROI. Mean T_2 values for corresponding ROI's are compared across imaging sessions to determine muscle activation status. This processing is currently performed using ANALYZE, but will be implemented in Matlab to incorporate additional statistical and display capabilities.

B. <u>Results:</u>

1. Design & implementation of the experimental protocol - We designed and constructed a lift and carry exercise ergometer [Figure 1 (ergometer) & Figure 2 (weighted box)] that successfully recruits a large portion of the total body's musculature. Figure 3 shows the ergometer and weighted box together. Initially the weighted box was fitted with a 2in dia. high molecular weight plastic rod, so as to more reliably mimic the military task of lifting and carrying an artillery shell. Our first subject was a 29 year old, 175lb Caucasian male of 5ft 11in height. The subject is a former member of the military who is currently in the National Guard, he is in superior physical condition and was well aquainted with scientific protocol. However in our initial MRI & exercise session, he was unable to pull the box close to his body, the result being that he experienced undue stress on his wrists and was unable to complete the protocol (stopped in the fifth of twelve 15min sessions). He noted that he was not particularly stressed anywhere but in his wrists. Following that first exercise session, the weighted box was refitted with a set of handles

mounted at 45° angles to the edge of the box (Figure 2 & Figure 3), a design that corresponds to that employed at USARIEM. This design has the advantage that data from these studies will be more directly comparable with studies done at USARIEM.

In a second exercise session, 1 month later, the same subject successfully completed the 6hr protocol without significant soreness in his wrists. Over the course of the session the subject first reported a tightness in his forearms in the fourth 15min session (45min-1hr of exercise). He became increasingly sloppy in his lifting form over the next eight 15min sessions (1-3hr of exercise). By the end of the session, he reported a general feeling of fatigue, with no specific focus of pain. When asked to try and locate a focus of pain, he responded that his shoulders were stiff and his hands were sore. At different times throughout the course of the protocol (3hr exercise in a 6hr period), the subject identified his forearms, his knees, his deltoids, his upper back, his biceps, and his neck as areas of soreness. The subject also reported a headache about halfway through the protocol that persisted to the end of the protocol, he suspected that the source of the headache was hunger. In keeping with the format established in the introduction of this report, MRI results and muscle recruitment will be reported in part 3 of this results section.

2. Validation of differential muscle recruitment - We observed three different results with three different degrees of knee flexion / extension, thereby demonstrating the ability of MR imaging to track differential muscle recruitment. With full knee extension (180°) MR images demonstrated primary recruitment of the gastrocnemius, measured as an increase in T_2 relaxation times. With the knee flexed at 90° the soleus was primarily recruited, with some secondary recruitment of the gastrocnemius. With the knee at an intermediate degree of flexion / extension (135°) both muscles were recruited. Table 1 (Appendix D) gives mean±SE changes in the gastrocnemius, the soleus, and the peroneus at three different degrees of knee flexion / extension. The muscles of the anterior compartment (anterior tibialis & extensors) were not included in the table because the only lower leg muscles recruited during this protocol were the three presented. Recruitment of the peroneus has been observed before, and is an indication of ankle eversion / inversion. This is the result of our ergometer design, which does not stabilize the ankle. The ankle must therefore be stabilized by the peroneus.

3. Development & implementation of total body MR imaging - The total body protocol was successfully implemented on one male subject (see section 1 of results). The MR images demonstrated significant recruitment of muscles of the lower body and of the

forearms. Figure 4 represents a single slice coronal reconstruction of 135 transverse images before and after fifteen minutes lift and carry exercise. Although grainy due to a combination of lack of resolution of the echo planar protocol and collection of the transverse images in a non-overlapping protocol, these total body images clearly demonstrate an increased signal intensity in the lower body and in the forearm region. When this data are processed as T_2 maps and ROI's are assayed within different muscles and muscle groups (150-350pixels per ROI), there are significant increases in the mean pixel T_2 's within several muscles (values given in Table 2, Appendix E). The T_2 changes are entered into a color coded anatomic map (front & rear view) at 1hr, 2hr, and 3hr exercise. This is presented along with the color codes corresponding increasing changes in T_2 values in Figures 5-7. This portion of our study, while incomplete, has been quite successful in its first iteration, clearly demonstrating the ability to differentiate muscle recruitment patterns in a series of total body MR images. The sheer magnitude of the data has resulted in a less than complete analysis of our subject's results, the data processing protocol is currently being upgraded.

C. Discussion:

The results of this first year of study have successfully demonstrated the capacity of the total body MR protocol to detect differential muscle recruitment patterns. The experimental design & the construction of the exercise ergometer supported a successful completion of a single study. While this first year of research could be viewed as a failure to accomplish the number of studies that was originally intended, the successfully streamed protocol lays a foundation for a rapid accumulation of data from this point on. The scout study employed conventional MR imaging to successfully demonstrate differential recruitment in a single group of muscles as a function of joint orientation. The ultimate result this work toward accomplishing the first specific aim of our project is that we have demonstrated that our male subject during performance of a prolonged military lift & carry task recruited much of his lower body and his upper arms & forearms.

The scout study of differential muscle recruitment (part 2) carries forward previous work that has reported the relationship between workload, exercise duration, and T_2 change (5,6,9,10). In an earlier studies we established a standardized exercise protocol that produced a similar T_2 increase in two muscles of vastly different sizes and strengths (6). The current work demonstrates that the degree of flexion / extension of the knee plays a role in the recruitment of the gastrocnemius versus the soleus in plantar flexion. To our knowledge, this is the first demonstration of the ability of MR imaging to track differential

muscle recruitment under different joint orientations. In Figure 8 we demonstrate the contribution of the major muscles of plantar flexion with different degrees of knee flexion / extension. Because this figure is speculative, and depends upon three assumptions that have not been scientifically demonstrated, it is presented in the discussion section. The figure relies on the following three assumptions: 1) plantar flexion is accomplished by only the soleus and the gastrocnemius and any additional muscles that are recruited (preonius) are working to accomplish other tasks (ankle stability), 2) The MVC of the soleus and the MVC of the gastrocnemius are roughly equivalent, and 3) the total increase in T_2 in the soleus and the gastrocnemius is a fair representation of the total plantar flexion work. When these assumptions are made, the figure demonstrates that during plantar flexion 90% of the work is done by the gastrocnemius at 180° knee angle, 80-90% of the work is done by the soleus at 90° knee angle, and at 135° knee angle the work is shared by the two muscles. When complete, this study of the lower leg will establish the basis for our current study of total body muscle recruitment.

D. Relationship to Statement of Work (SOW) outlined in the proposal:

These results have little relationship with the original SOW, and we have been in ongoing conversation with Brian Martin and Patricia Modrow since the summer to restructure the SOW and rebudget the contract. The conversation has recently come to fruition and a revised budget and scientific structure are in place. The results in this report remain behind the revised SOW; however, the recent addition of a computer engineer and a research assistant are expected to have the project on track by the end of the second year. There have been a number of revisions in the way that we expect to gather both MRI and MRS data that, while not having a great effect now, will have the enable data to be gathered at a greatly enhanced rate during the remainder of the project.

E. Negative results:

The major negative result of this first year of our project was the initial failure of our lone subject to accomplish the protocol. This resulted from an inadequate design of the method of lifting the weighted box. The problem was overcome by redesigning the weighted box to more closely comply with the proven design used at USARIEM. The bulk of data that is generated in a single total body experiment has been a difficulty; however, with the addition of a computer engineer to our staff we have made great strides in the last

four months, and we are now in a position to handle the incoming data reliably. This is largely due to the purchase of data storage media and data a couple of dedicated data processing computers.

F. Problems in accomplishing tasks:

The major problem that we have encountered with this project has been in recruiting subjects that are physically capable of performing our exercise protocol. While we have yet to accomplish the protocol with a female subject, we have three female subjects that currently in the screening process. There is some concern about the 65lb mass and the ability of female subjects to complete the task; however, we have scheduled the strongest female candidate to perform the initial study in the female group. If she is unable to accomplish the task, we will have to consider reducing the mass of the weighted box. The recent success that we have had in subject recruitment is due to the addition of a research assistant, who is primarily used to recruit subjects, to our staff.

CONCLUSIONS

From this first year of our project we conclude that the experimental protocol is adequate to accomplish the remainder of the project. While there are concerns about the mass that will ultimately be used to compare men and women, we have no doubt that we will be able to determine a mass the reliably compares men & women under our specific conditions using our methodologies. We conclude from the scout study that MR imaging provides a viable tool to assess differential muscle recruitment patterns. We conclude from the total body MR imaging that we will be able to reliably assess systemic muscle recruitment patterns during prolonged exercise. Furthermore, the range of T_2 changes seen in our initial study suggests that muscles that are working harder to perform a task can be identified. Given the differences in mean lifting strength between men and women, we expect to be able to differentiate between genders with total body MRI.

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FIGURE 1: Lift and carry exercise ergometer, designed to deliver weighted box from 52in height to floor level.

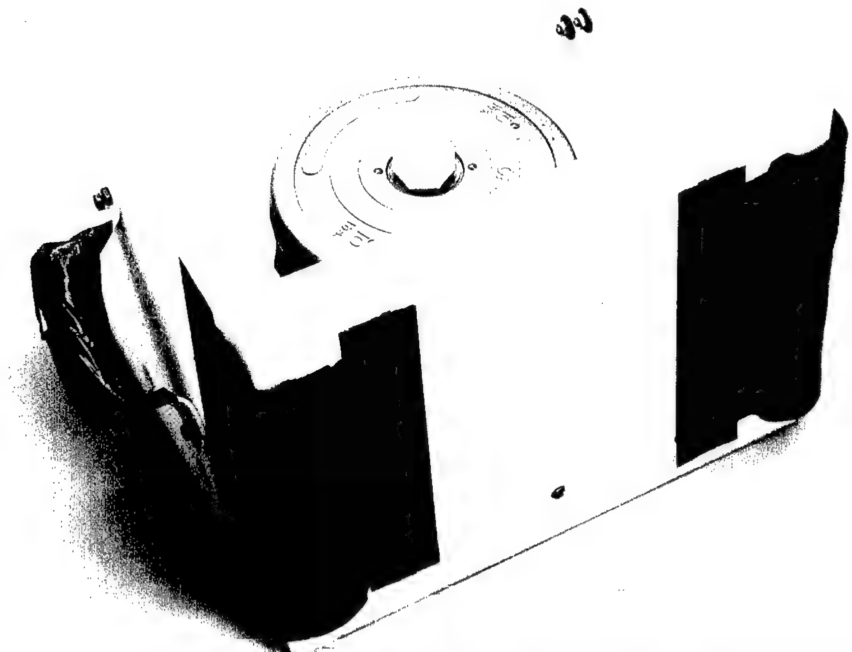


FIGURE 2: Weighted box (45lb) with 20lb added weight.

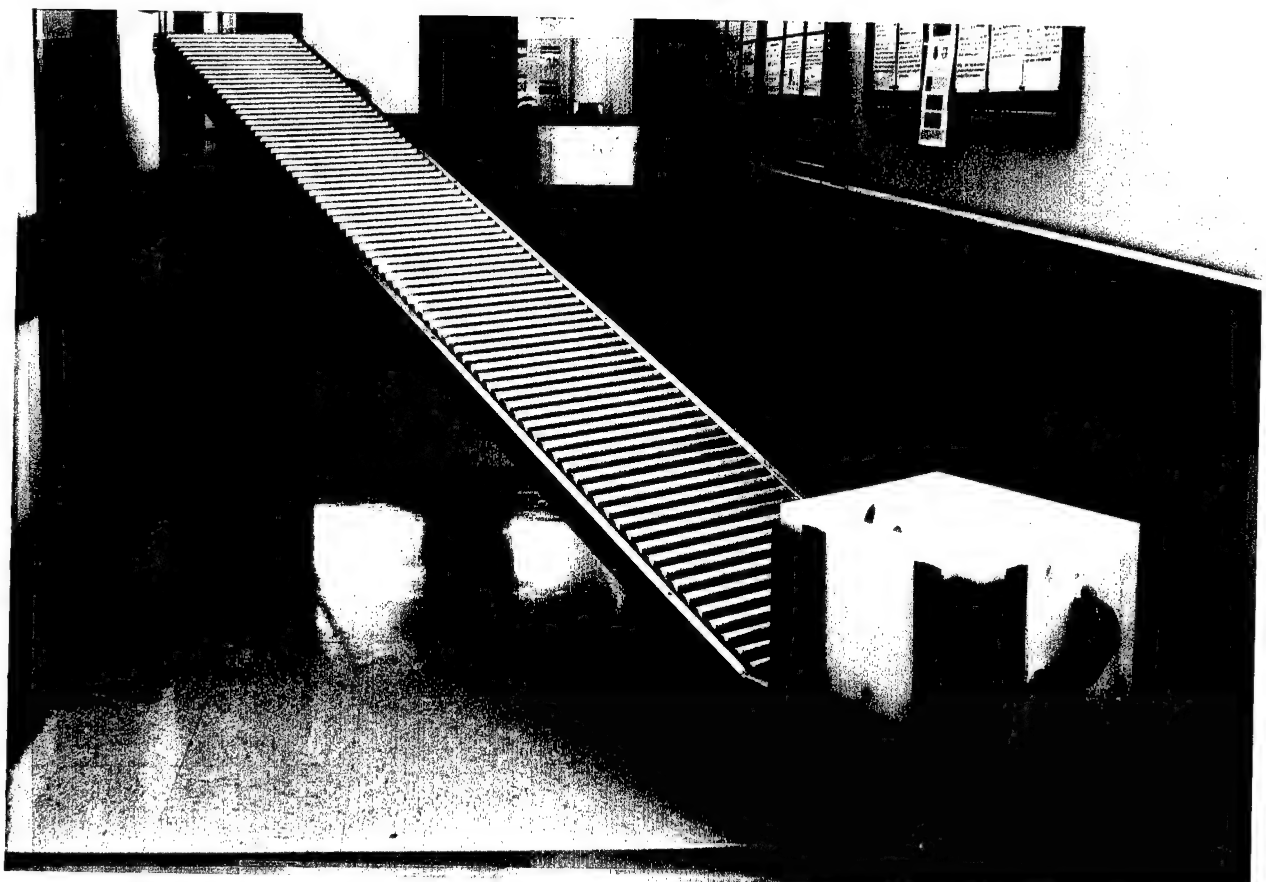
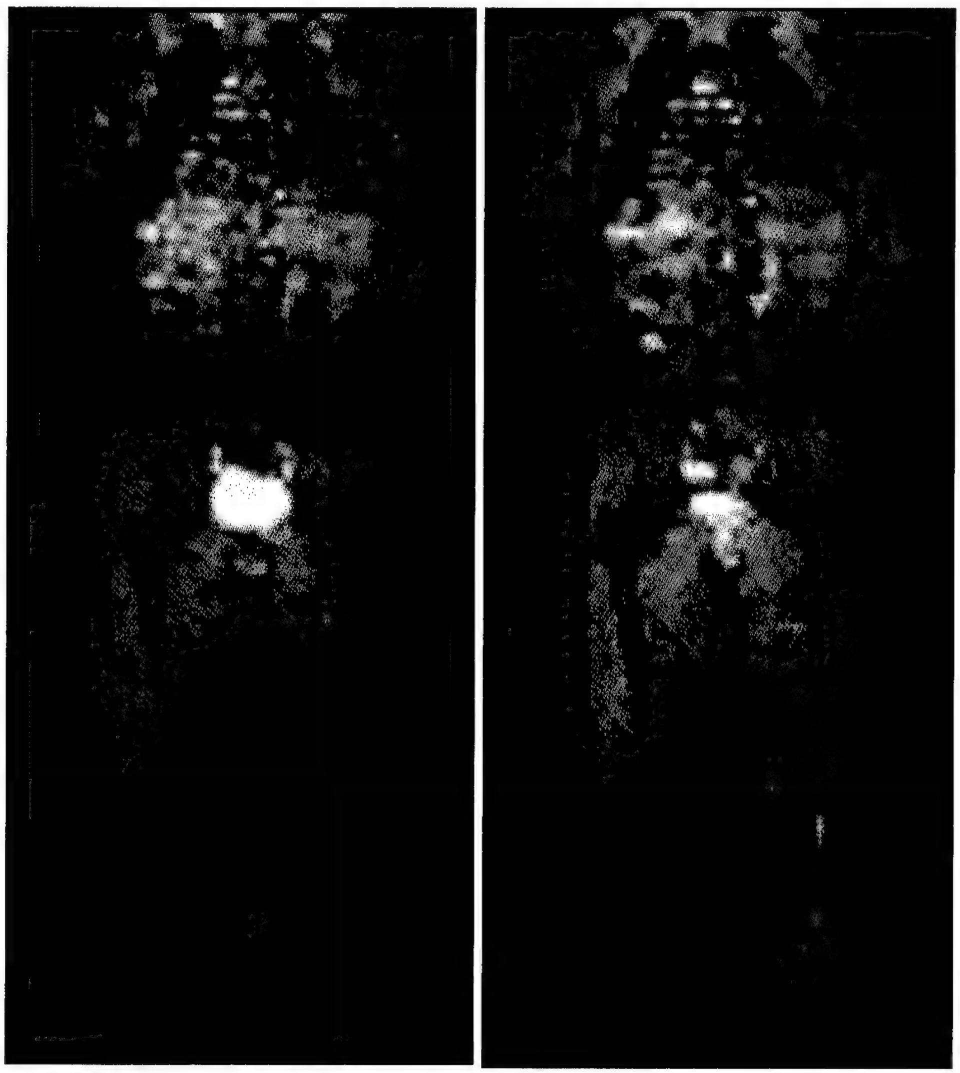


FIGURE 3: Lift & carry ergometer with weighted box.

FIGURE 4: Single slice coronal reconstruction of 135 transverse images before and after a single 15min bout of exercise.



BEFORE EXERCISE AFTER EXERCISE

FIGURE 5: Color map, color is coded to the amount of increase in T_2 time following exercise.

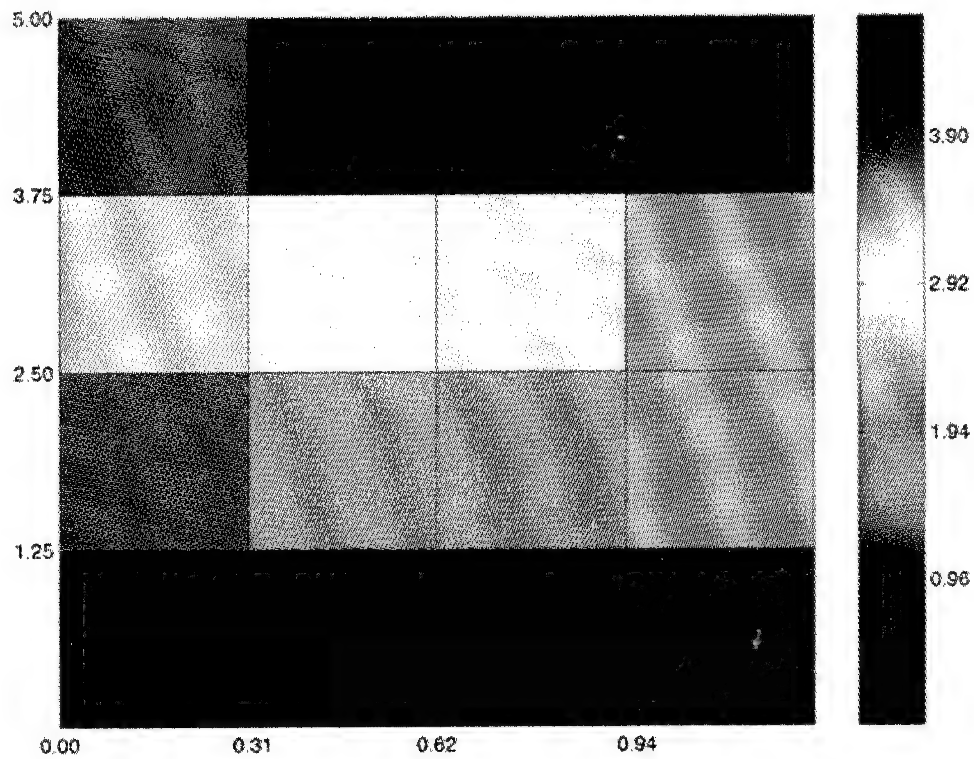
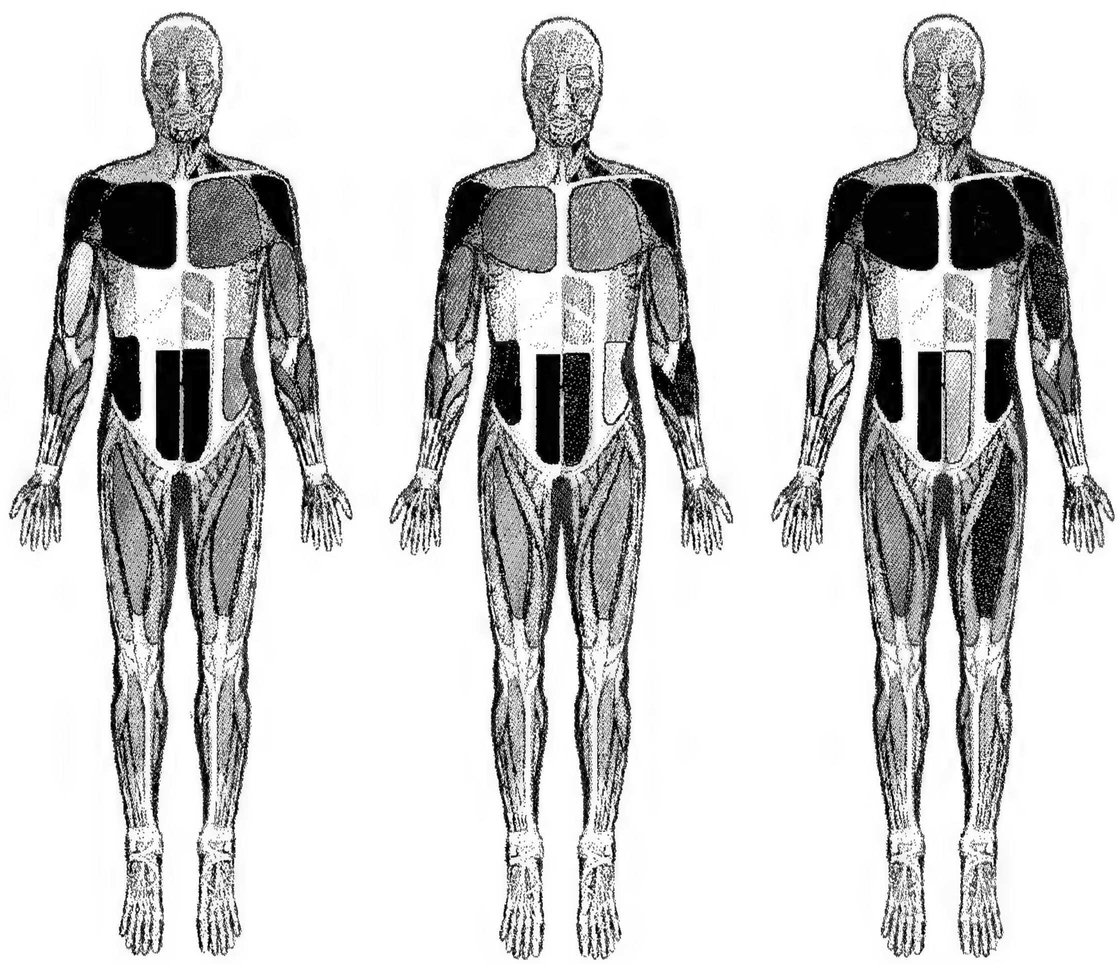
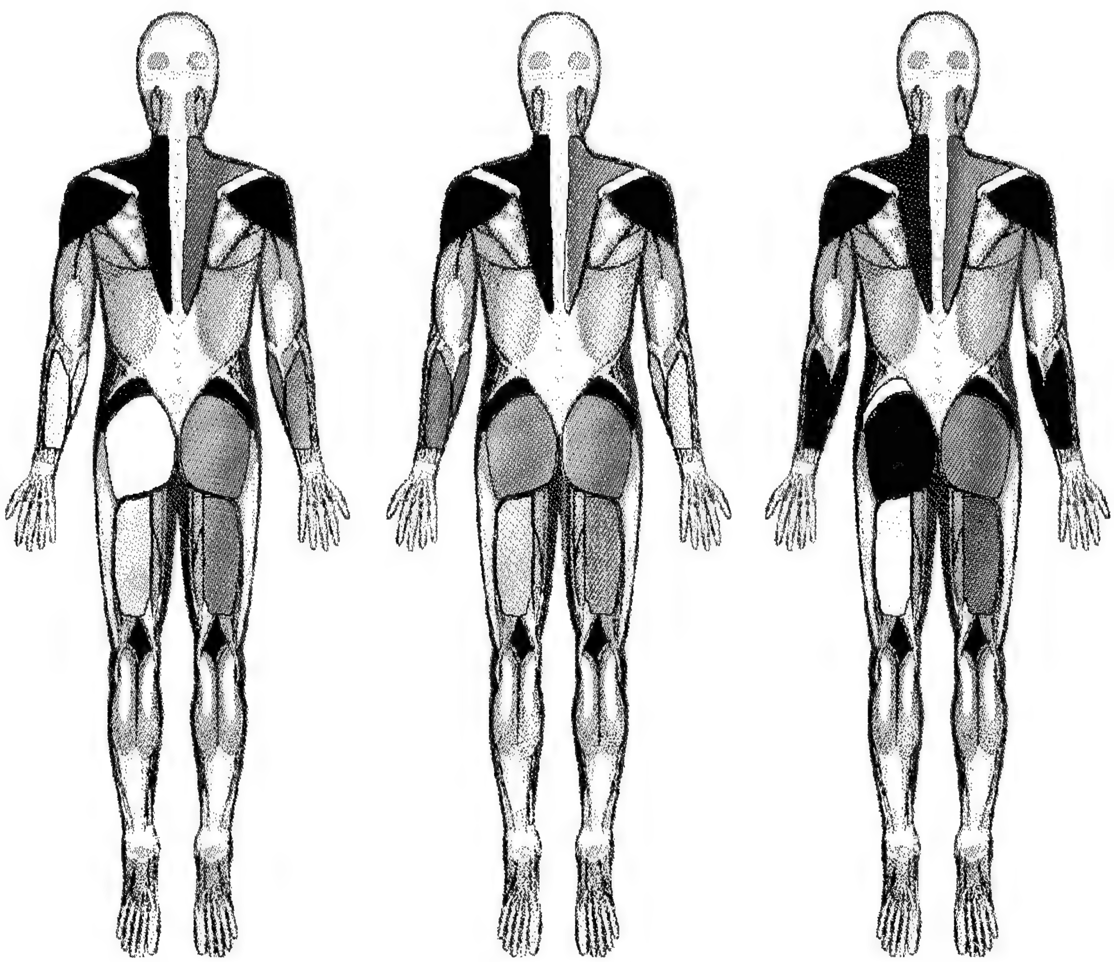


FIGURE 6: Front view of male subject with exercised muscles color coded according to the amount of increase in T_2 time.



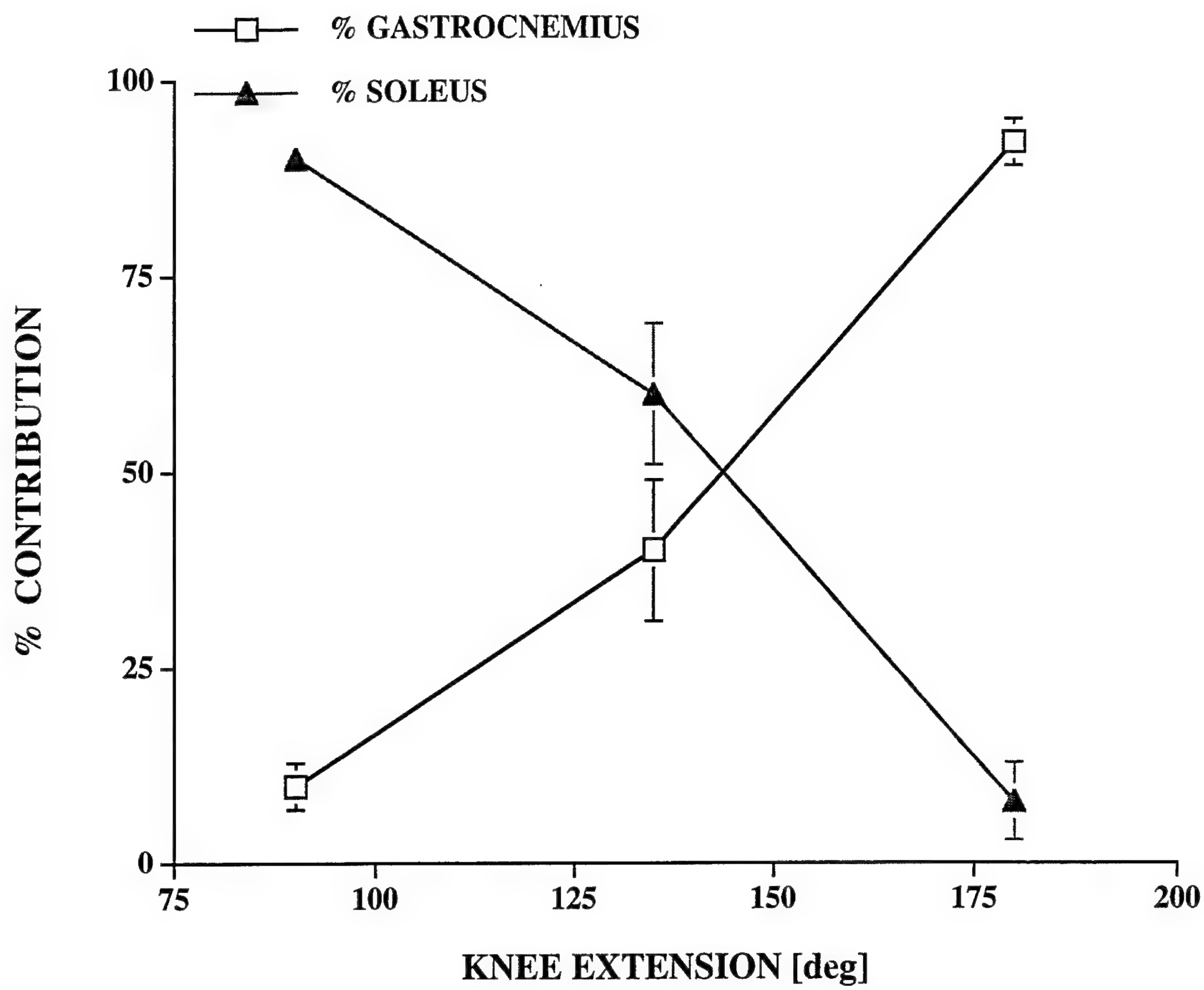
1hr EXERCISE 2hr EXERCISE 3hr EXERCISE

FIGURE 7: Back view of male subject with exercised muscles color coded according to the amount of increase in T_2 time.



1hr EXERCISE 2hr EXERCISE 3hr EXERCISE

FIGURE 8: % contribution of the gastrocnemius and the soleus to plantar flexion at three different degrees of knee flexion / extension.



APPENDIX A: Subject recruitment information forms

Personal habits:

SMOKING:____yes____no

if: yes then: Packs per day:__________ # of years:________________

if: quit then: When:____________

ALCOHOL CONSUMPTION:____yes____no

if: yes then: Drinks per day__________ # of years:________________

Basic medical:

ALLERGIES:____none____dust____animals____hay fever____penicillin

____asprin____Tylenol____other

Type of allergic reaction:____

MEDICATIONS: ____none____medication

MEDICAL CONDITIONS:____none__________________________________condition

FAMILY HISTORY OF DIABETES:____none__________type_______age of onset

treatment:_______________________

check one: ____mother____father____sister____brother

____maternal grandfather____maternal grandmother

____paternal grandfather____paternal grandmother

SURGERY:____none____surgery

CLAUSTROPHOBIA:____yes____no

Research History:

RESEARCH EXPERIENCE:____none____psychological____medical

____MRI / MRS____iv

METAL IMPLANTS:____yes____no

Schedule Flexibility:

AVAILABLE ALL DAY:___M___Tu___W___Th___F___Sa___Su (all that apply)

screen date____/____/____ study date____/____/____

diet starts____/____/____

APPENDIX A: Subject recruitment information forms

BASIC DATA SCREENING SHEET:

Basic information:

NAME:_______________________________________last, first, m.i.

ADDRESS:____

____

TELEPHONE: (   )____-_______ home (   )____-_______ work

(   )____-_______ FAX ______________________ e-mail

SOCIAL SECURITY #: _______________

OCCUPATION: ____

Address: ____

____

MARITAL STATUS: ______ married _______ single

Number of children _______________

Personal information:

AGE:_________ Date of Birth:_____/_____/_____

WEIGHT:____________

HEIGHT:____________

ETHNIC ORGIN:____caucasian____african american____hispanic

____east asian____other

SEX:____female____male

if: female then: Birth Control:____yes____no

if: yes then: Type of birth control:___________________________

How long? ____________

MENSTRUAL HISTORY:

APPENDIX A: Subject recruitment information forms

EXERCISE DATA SCREENING SHEET:

NAME________________________________AGE____DATE____/____/____

Approximately how many sessions per week during the past MONTH have you engaged in the following activities? (CIRCLE ONE EA. CATEGORY THAT APPLIES)

	SESSIONS								
running	1	2	3	4	5	6	7	8	9+
swimming	1	2	3	4	5	6	7	8	9+
bicycling	1	2	3	4	5	6	7	8	9+
walking	1	2	3	4	5	6	7	8	9+
aerobics	1	2	3	4	5	6	7	8	9+
weight lifting	1	2	3	4	5	6	7	8	9+
calisthenics	1	2	3	4	5	6	7	8	9+
team sports (softball,etc)	1	2	3	4	5	6	7	8	9+
stair climber	1	2	3	4	5	6	7	8	9+
other__________ ______________	1	2	3	4	5	6	7	8	9+

Approximately how many minutes do you spend doing this activity during one workout period? (CIRCLE ONE EA. CATEGORY THAT APPLIES)

MINUTES PER WORKOUT

	10	20	30	40	50	60	70	80	90+
running	1	2	3	4	5	6	7	8	9+
swimming	1	2	3	4	5	6	7	8	9+
bicycling	1	2	3	4	5	6	7	8	9+
walking	1	2	3	4	5	6	7	8	9+
aerobics	1	2	3	4	5	6	7	8	9+
weight lifting	1	2	3	4	5	6	7	8	9+
calisthenics	1	2	3	4	5	6	7	8	9+
team sports (softball,etc)	1	2	3	4	5	6	7	8	9+
stair climber	1	2	3	4	5	6	7	8	9+
other__________ ______________	1	2	3	4	5	6	7	8	9+

Approximately how long have you been on this schedule? (CIRCLE ONE EA. CATEGORY THAT APPLIES)

MONTHS

running	1	2	3	4	5	6	7	8	9+
swimming	1	2	3	4	5	6	7	8	9+
bicycling	1	2	3	4	5	6	7	8	9+
walking	1	2	3	4	5	6	7	8	9+
aerobics	1	2	3	4	5	6	7	8	9+
weight lifting	1	2	3	4	5	6	7	8	9+
calisthenics	1	2	3	4	5	6	7	8	9+
team sports (softball,etc)	1	2	3	4	5	6	7	8	9+
stair climber	1	2	3	4	5	6	7	8	9+
other__________ ______________	1	2	3	4	5	6	7	8	9+

How many times a week do you participate in each activity at the following intensities?

VLOWLOW **MOD** **HIGH** **VHIGH**

running _____ _____ _____ _____ _____
swimming _____ _____ _____ _____ _____ bicycling _____
_____ _____ _____ _____ walking _____ _____ _____ _____
_____ aerobics _____ _____ _____ _____ _____ weight lifting
_____ _____ _____ _____ _____ calisthenics _____ _____ _____
_____ _____ team sports
(softball,etc)
stair climber _____ _____ _____ _____ _____ other__________ _____
_____ _____ _____ _____ ______________

If you lift weights, do you regularly (every week) perform (check):

arm curls ____yes____no
arm extensions ____yes____no
squats ____yes____no
bench press ____yes____no
decline press____yes____no
incline press ____yes____no
leg press ____yes____no
military press____yes____no
leg curl ____yes____no
leg extension____yes____no
lat pull ____yes____no
plantar flexes____yes____no

If you regularly participate in a walking or running exercise program, what percentage (on the average) of your workout is:

UPHILL ________%
LEVEL ________%
DOWNHILL ________%

Do you spend time stretching before and after a workout?

	NEVER	RARELY	SOMETIMES	OFTEN	ALWAYS
BEFORE	____	____	____	____	____
AFTER	____	____	____	____	____

How much time is spent stretching?

BEFORE __________minutes
AFTER __________minutes

APPENDIX A: Subject recruitment information forms

NUTRITIONAL DATA SCREENING SHEET:

What do you typically eat ?

WEEKDAYS:

breakfast____

lunch____

dinner____

snacks (between meals daytime)______________________________

snacks (after 8:30 PM - be honest)______________________________

WEEKENDS:

breakfast____

lunch____

dinner____

snacks (between meals daytime)______________________________

snacks (after 8:30 PM - be honest)______________________________

Do you have any food allergies or intolerances?

____none____allergies

____intolerances

Are you currently on, or have you previously been on, a diet?

____yes____no

if: yes then: ____type of diet

if: you are no longer on the diet then: when did you stop the diet?____________

Are you, or have you ever been, a vegetarian? ____yes____no

if: yes then: ____description

if: you were a vegetarian then: how long since you stopped________________

How long, typically, does it take for you to eat a meal? __________minutes

When you cook a meal for yourself, is it typically:

BAKED ____yes____no _________% time

BROILED ____yes____no _________% time

FRIED ____yes____no _________% time

MICROWAVED ____yes____no _________% time

Do you usually:

EAT OUT ____yes____no _________% time

EAT AT HOME ____yes____no _________% time

How often (during the day & evening) do you eat?

number of times_________

APPENDIX B: Weekly checklist (diet & exercise) during month before study

WEEKLY CHECKLIST (WEEK 1)

EXERCISE (number of sessions, low long each session)

running______ , ______ swimming______ , ______ bicycling______ , ______

aerobics______ , ______ weights______ , ______ calisthenics______ , ______

team sports (softball,etc)______ , ______ stair climber ______ , ______

other (list)______________________ , number______ , how long______

DIET (what you ate)

1 breakfast____

lunch____

dinner____

2 breakfast____

lunch____

dinner____

3 breakfast____

lunch____

dinner____

4 breakfast____

lunch____

dinner____

5 breakfast____

lunch____

dinner____

6 breakfast____

lunch____

dinner____

7 breakfast____

lunch____

dinner____

2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,15min RUN (dist)______

2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,15min RUN (dist)______

FEMALE SUBJECTS (week 1): **take body temperature when you wake up**
DAILY BODY TEMPERATURE (time,temp) DAY 1 ____ , ____ 2 ____ , ____
3 ____ , ____ 4 ____ , ____ 5 ____ , ____ 6 ____ , ____ 7 ____ , ____

BLOOD SAMPLES (1) progesterone ____ , estradiol ____
(2) progesterone ____ , estradiol ____

FEMALE SUBJECTS (week 2): **take body temperature when you wake up**
DAILY BODY TEMPERATURE (time,temp) DAY 1 ____ , ____ 2 ____ , ____
3 ____ , ____ 4 ____ , ____ 5 ____ , ____ 6 ____ , ____ 7 ____ , ____

BLOOD SAMPLES (1) progesterone ____ , estradiol ____
(2) progesterone ____ , estradiol ____

FEMALE SUBJECTS (week 3): **take body temperature when you wake up**
DAILY BODY TEMPERATURE (time,temp) DAY 1 ____ , ____ 2 ____ , ____
3 ____ , ____ 4 ____ , ____ 5 ____ , ____ 6 ____ , ____ 7 ____ , ____

BLOOD SAMPLES (1) progesterone ____ , estradiol ____
(2) progesterone ____ , estradiol ____

FEMALE SUBJECTS (week 4): **take body temperature when you wake up**
DAILY BODY TEMPERATURE (time,temp) DAY 1 ____ , ____ 2 ____ , ____
3 ____ , ____ 4 ____ , ____ 5 ____ , ____ 6 ____ , ____ 7 ____ , ____

BLOOD SAMPLES (1) progesterone ____ , estradiol ____
(2) progesterone ____ , estradiol ____

APPENDIX B: Weekly checklist (diet & exercise) during month before study

WEEKLY CHECKLIST (WEEK 2)

EXERCISE (number of sessions, low long each session)
running______ , ______ swimming______ , ______ bicycling______ , ______
aerobics______ , ______ weights______ , ______ calisthenics______ , ______
team sports (softball,etc)______ , ______ stair climber ______ , ______
other (list)____________________ , number______ , how long______

DIET (what you ate)
1 breakfast______________________________
lunch______________________________
dinner______________________________
2 breakfast______________________________
lunch______________________________
dinner______________________________
3 breakfast______________________________
lunch______________________________
dinner______________________________
4 breakfast______________________________
lunch______________________________
dinner______________________________
5 breakfast______________________________
lunch______________________________
dinner______________________________
6 breakfast______________________________
lunch______________________________
dinner______________________________
7 breakfast______________________________
lunch______________________________
dinner______________________________

2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,15min RUN (dist)______
2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,15min RUN (dist)______

BODY COMPOSITION TEST (skinfold thickness) ______ %BF ________

WEEKLY CHECKLIST (WEEK 3)

EXERCISE (number of sessions, low long each session)
running______ , ______ swimming______ , ______ bicycling______ , ______
aerobics______ , ______ weights______ , ______ calisthenics______ , ______
team sports (softball,etc)______ , ______ stair climber ______ , ______
other (list)______________________ , number______ , how long______

DIET (what you ate)
1 breakfast____
lunch____
dinner____
2 breakfast____
lunch____
dinner____
3 breakfast____
lunch____
dinner____
4 breakfast____
lunch____
dinner____
5 breakfast____
lunch____
dinner____
6 breakfast____
lunch____
dinner____
7 breakfast____
lunch____
dinner____

2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,15min RUN (dist)______
2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,15min RUN (dist)______
EXERCISE TRAINING SESSION ______
VO2max ______________ per kg , MAXIMUM HEARTRATE __________bpm

APPENDIX B: Weekly checklist (diet & exercise) during month before study

WEEKLY CHECKLIST (WEEK 4)

EXERCISE (number of sessions, low long each session)

running______ , ______ swimming______ , ______ bicycling______ , ______

aerobics______ , ______ weights______ , ______ calisthenics______ , ______

team sports (softball,etc)______ , ______ stair climber ______ , ______

other (list)______________________ , number______ , how long______

DIET (what you ate)

WHAT PERCENT OF THE STANDARD DIET DID YOU EAT?

DAY 1 ________ , DAY 2 ________ , DAY 3 ________ , DAY 4 ________ ,

DAY 5 ________ , DAY 6 ________ , DAY 7 ________

2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,15min RUN (dist)______

<u>ARMY PHYSICAL READINESS TEST:</u>

2min PUSH-UPS (#)______ ,2min SIT-UPS (#)______ ,2mi. RUN (min)______

APPENDIX C: Monthly checklist (meals, exercise, & screening tests)

SUBJECT____________________

SEX _________

MONTH PREPARATION PROTOCOL CHECKLIST

	WEEK 1	WEEK2	WEEK3	WEEK 4
DAILY EXERCISE	_ _ _ _ _ _ _	_ _ _ _ _ _ _	_ _ _ _ _ _ _	_ _ _ _ _ _ _
DAILY MEALS	_ _ _ _ _ _ _	_ _ _ _ _ _ _	_ _ _ _ _ _ _	_ _ _ _ _ _ _
PUSH-UP/SIT-UP/ RUN (SESSION 1)	______	______	______	______
PUSH-UP/SIT-UP/ RUN (SESSION 2)	______	______	______	______
SKINFOLD THICKNESS	______	______	______	______
EXERCISE TRAINING SESSION	______	______	______	______
VO_{2max} TEST	______	______	______	______
ARMY PRT	______	______	______	______
SPECIAL DIET	______	______	______	______

APPENDIX D:

Table 1: Change in T_2 in the soleus, gastrocnemius, and the peroneus at 90°, 135°, and 180° knee angle following 5min plantar flexion at 25% of MVC. Values are mean ± SE. (n = 3) * = p≤0.05 relative to 90°. ** = p≤0.05 relative to gastrocnemius at same knee angle.

CHANGE IN T_2 [msec]

	GASTROGNEMIUS	SOLEUS	PERONEUS
90°	0.3 ± 0.4 ms	4.0 ± 1.2 ms**	1.6 ± 0.1 ms**
135°	4.7 ± 2.6 ms	5.1 ± 1.6 ms	4.0 ± 2.0 ms
180°	8.8 ±2.4 ms *	1.0 ± 0.5 ms**	6.4 ± 1.9 ms

APPENDIX E:

Table 2a: Degree of significance of muscle recruitment in contralateral (R & L) muscles of the upper body at different timepoints during a 3hr exercise protocol performed over a 6hr period.

	Trapezius		Deltoids		Biceps		Sup.Forearm		Inf.Forearm		Pect.Major		Pect. Minor	
TIME [hr]	R	L	R	L	R	L	R	L	R	L	R	L	R	L
0.25	0.01	---	---	---	0.001	0.05	0.001	0.001	0.001	0.001	0.001	0.001	---	---
0.5	0.001	---	0.02	---	0.001	0.001	0.001	0.001	0.001	0.001	---	---	---	---
0.75	0.001	---	---	---	0.001	0.01	0.001	0.001	0.001	0.001				
1	0.02	---	---	---	0.001	0.001	0.001	0.001	0.001	0.001	---	0.01	---	---
1.25	0.02	---	---	---	---	0.001	0.001	0.001	0.001	0.001				
1.5	0.01	---	---	---	0.05	0.05	0.001	0.001	0.001	0.001	0.01	0.05	0.02	---
1.75	0.02	---	---	---	0.02	0.05	0.001	0.001	0.001	0.001				
2	0.01	---	---	---	0.001	0.01	0.001	0.001	0.001	0.001	0.001	0.01	---	---
2.25	0.01	---	---	---	0.01	---	0.001	0.001	0.001	0.001				
2.5	0.01	---	---	---	0.05	0.05	0.001	0.001	0.001	0.001	---	---	---	---
2.75	0.01	---	---	---	0.001	0.05	0.001	0.001	0.001	0.001				
3	0.001	---	---	---	0.001	0.02	0.001	0.001	0.001	0.001	0.01	0.05	---	---

Table 2b: Degree of significance of muscle recruitment in contralateral (R & L) muscles of the trunk at different timepoints during a 3hr exercise protocol performed over a 6hr period.

	Rectus Abdominus		External Lateral Obliques	
TIME [hr]	R	L	R	L
0.25	---	---	0.001	---
0.5	---	---	---	0.001
0.75				
1	---	---	0.01	0.001
1.25				
1.5	---	---	0.02	0.001
1.75				
2	---	0.001	---	0.001
2.25				
2.5	---	0.02	---	0.001
2.75				
3	---	0.001	0.001	0.001

APPENDIX E:

Table 2c: Degree of significance of muscle recruitment in contralateral (R & L) muscles of the lower body at different timepoints during a 3hr exercise protocol performed over a 6hr period.

	Gluteus Maximus		Gluteus Medius		Quadriceps		Hamstrings	
TIME [hr]	R	L	R	L	R	L	R	L
0.25	0.001	0.001	---	0.001	0.001	0.02	0.001	0.001
0.5	0.02	0.001	---	0.001	0.01	0.02	0.01	0.001
0.75					0.01	0.05	0.001	0.001
1	0.05	0.001	---	0.001	0.001	0.001	0.001	0.001
1.25					0.001	0.001	0.001	0.001
1.5	---	0.02	---	0.001	0.001	0.001	0.001	0.001
1.75					0.001	0.001	0.001	0.001
2	0.01	0.001	---	0.001	0.001	0.02	0.01	0.001
2.25					0.01	0.01	0.05	0.001
2.5	---	---	---	0.001	0.001	---	0.001	0.001
2.75					---	0.05	0.02	0.001
3	0.001	---	---	0.001	0.001	---	0.05	0.001